We believe that these cells are the cells of origin of the SP-like immunoreactive fibers entering the frog spinal cord through the dorsal root and distributing to the dorsal horn[6].
The SP-like immunoreactive fibers, before reaching the place where central and peripheral roots emerge from the spinal ganglion, collect in small bundles of several fibers within the ganglion neuropil (figure, a and d). This suggests that cells containing the same putative transmitter have their processes assembled together and may maintain their group individuality when they run in the roots where other fibers with other neurotransmitters are also running.
Our results demonstrate that substance P is present in approximately 50% of the population of primary sensory neurons in thoracic DRG of the frog.

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Changes in brush-border enzyme activities of intestinal epithelial cells isolated from the villus-crypt axis during the early phase of alloxan diabetes in rats

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Summary. The sucrase activity in enterocytes isolated from the villus crypt axis was found to increase in all regions of the villus from day 2 after induction of diabetes, and the increase continued until day 4. In contrast, alkaline phosphatase activity increased mainly in the apical one-third of the villus-crypt column, and the increase occurred abruptly on day 4 with increase in food intake.
Key words. Rat; alloxan diabetes; intestinal villus; alkaline phosphatase; sucrase; epithelial cells; villus-crypt axis.

The activities of hydrolytic enzymes in the intestinal brush-border membrane are known to be increased in diabetic animals[1-5]. However, the site of these increased activities along the villus-crypt axis of diabetic animals has not been investigated. In this study, we investigated the sites and time-courses of increase in alkaline phosphatase and sucrase activities in enterocytes from the villus-crypt axis during the early phase of induction of alloxan diabetes in rats.
Materials and methods. Male Sprague-Dawley rats weighing 160–190 g were kept in individual stainless-steel cages in an air-conditioned room at 23 ± 2 °C with lighting from 08.00 to 20.00 h. Experimental diabetes was induced by intramuscular injection of 120 mg of alloxan monohydrate (Wako Junyaku Co., Osaka, Japan) per kg b.wt (4% solution in saline). Control rats received an injection of saline instead. Food and water were freely available except in some experiments, in which from days 3 to 5 after alloxan treatment diabetic rats were given a restricted diet, equivalent in amount to that consumed by control rats.
The semisynthetic diet consisted of 20% casein, 45% α-corn starch, 23% sucrose, 5% oil (soybean oil:cod liver oil, 4:1, v/v), 4% salt mixture[6], 1% vitamin mixture[6], 1.85% cellulose powder (40–100 mesh), and 0.15% choline-Cl. Food intake and b.wt were recorded between 09.00 and 10.00 h every day.
All experiments were started at 10.00 h to avoid circadian changes[7]. Blood was withdrawn from the inferior vena cava of rats under ether anesthesia, and then the animals were killed by decapitation. The entire small intestine was quickly removed, rinsed thoroughly with ice-cold saline and divided into eight equal lengths. The second segment from the pyrolus was everted and its enterocytes were isolated along the villus-crypt axis by the methods of Weiser[8] and Raul et al.[9], except that the incubation times used were 10, 3, 4, 5, 6, 7, 8, 9, 10, 15 and 20 min with shaking (100 oscillations per min). Alkaline phosphatase was assayed by the method of Forstner et al.[10]. Sucrase activity was determined by the method of Dahlqvist[11]. Protein was measured by the method of Lowry et al.[12] with bovine serum albumin as a standard. Enzyme activities were expressed as amounts of substrates hydrolyzed per mg protein per min at 37 °C in μmoles for alkaline phosphatase, and in nmoles for sucrase. Blood sugar was determined by the method of Ashwell[13]. Animals with a plasma glucose level of more than 250 mg per 100 ml of plasma were regarded as diabetic. Data were analyzed by Student's t-test and a value of $p < 0.05$ was considered as significant.
Results and discussion. The gradients from the tip of the villus to the crypt of alkaline phosphatase and ^{3}H-thymidine incorporation after a 3-h pulse in vivo were examined by reported methods[8,9].
As shown in figure 1, alkaline phosphatase activity had decreased in all regions of the villus on the day after induction of diabetes. Food intake of the diabetic rats also decreased for the first few days after alloxan treatment. During this period, the activity of alkaline phosphatase remained low. Sucrase activity began to increase on day 2 and continued to increase until day 4 in the entire villus-crypt column. In contrast, alkaline phosphatase activity increased in the apical one-third of the villus-crypt column on day 4, when the diabetic rats became hyperphagic. Thus the localizations of the increased activities of alkaline phosphatase and sucrase along the villus-crypt axis of diabetic rats were different.
The times of increase in the activities of alkaline phosphatase

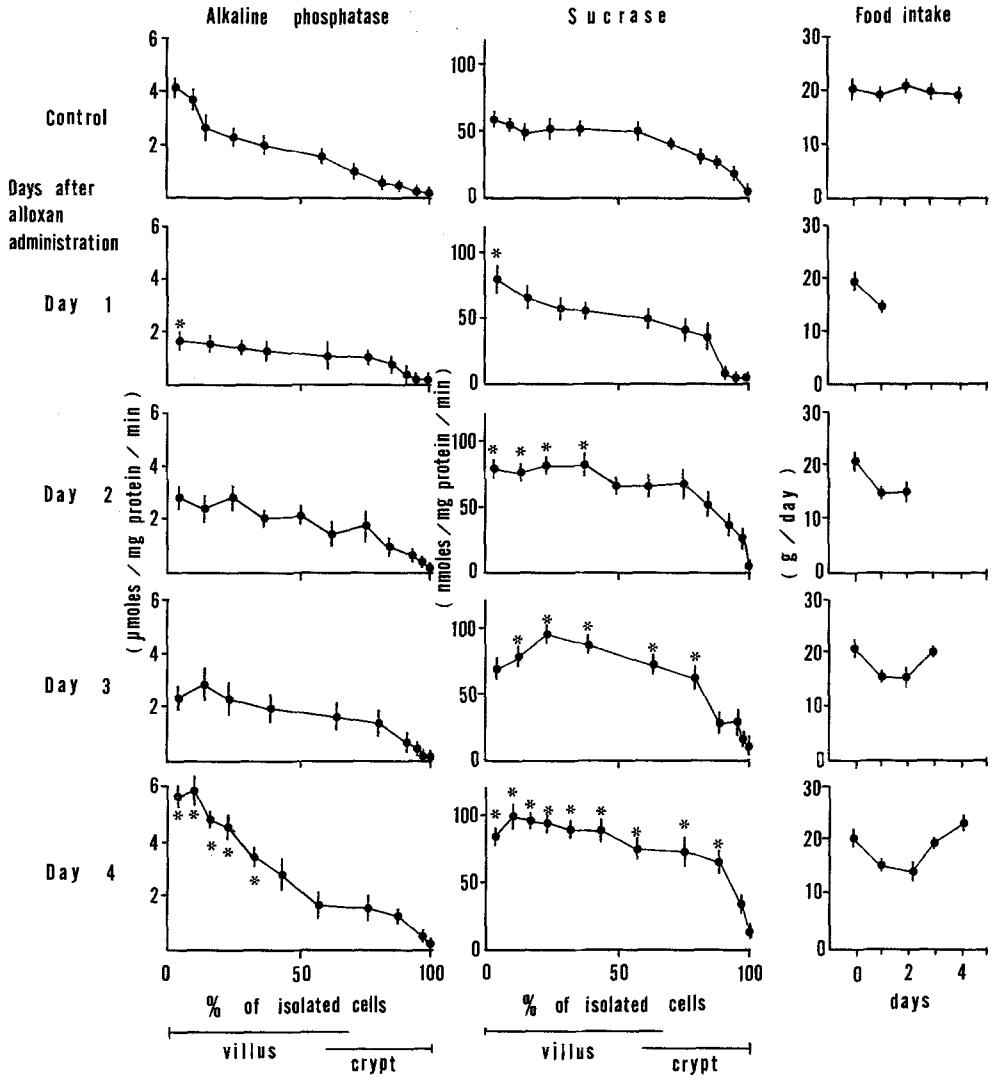

Figure 1. Changes in patterns of alkaline phosphatase and sucrase activities in epithelial cells along the villus-crypt axis of the small intestine of rats during the early stage of alloxan diabetes. Enzyme activities are expressed as µmoles or nmoles of substrate hydrolyzed per mg protein per min at 37 °C. The abscissa shows the percentages of cells isolated in successive fractions determined from the amount of cellular protein isolated in each fraction. Values are means ± SEM for 4–6 rats per group. *Significantly different from control: $p < 0.05$.

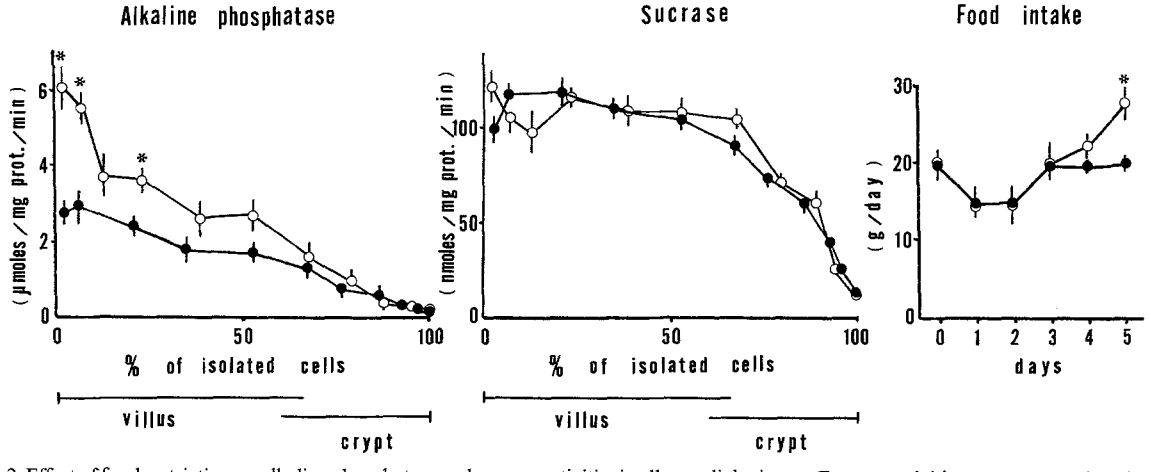

Figure 2. Effect of food restriction on alkaline phosphatase and sucrase activities in alloxan diabetic rats. Enzyme activities were measured on day 5 after alloxan treatment. ○ Diabetic rats fed ad libitum; ● diabetic rats given a restricted diet equivalent in amount to that consumed control rats, on the basis of daily intake, from day 3 to day 5 after alloxan treatment. Values are means ± SEM for five rats. *Significantly different from value for diabetic rats which were given a restricted diet ($p < 0.05$). For details, see figure 1 and 'Materials and methods'.

and sucrase also differed. This is in agreement with findings[14] on duodenal mucosa scrapings from diabetic mice. The different responses of these two enzymes after alloxan treatment are probably due to differences in their mechanisms of induction. Previously, we reported that in the chronic phase of diabetic rats[15], there is a positive correlation between food intake and alkaline phosphatase activity, but not disaccharidase activities.

To confirm the action of the effect of food consumption on enzyme induction, some diabetic rats were given restricted food, equivalent in amount to that consumed by control rats, from day 3 to 5. As shown in figure 2, on day 5, alkaline phosphatase did not increase in these animals, whereas the sucrase activity increased as in diabetic rats fed ad libitum. These results suggest that the induction of alkaline phosphatase activity in the diabetic intestine is caused by an increase in food intake resulting from insulin deficiency, whereas the induction of sucrase activity is caused by insulin deficiency in itself, or a humoral imbalance due to insulin deficiency.

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NADP-isocitric dehydrogenase of gerbil adrenal mitochondria: support of steroid hydroxylation

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Summary. In gerbil adrenal cortex the activity of intramitochondrial NADP-linked isocitric dehydrogenase (IDH) is up to 10-fold greater than the NAD-linked IDH. The NADP-IDH, apparent K_m 0.58 mM, V_{max} 280 nmoles/min/mg mitochondrial protein, appears to be the major source of reducing equivalents to support adrenal mitochondrial steroid 11B- and 19-hydroxylation in this species.

Key words. Gerbil, adrenal cortex; isocitric dehydrogenase; mitochondria; steroid; steroid hydroxylation.

Adrenal mitochondria contain cytochrome P-450 hemoproteins, cholesterol side chain cleavage and 11β-hydroxylase enzymes, which utilize oxygen and reducing equivalents transferred from NADPH to introduce hydroxyl groups into steroid hormone precursors[2]. Tricarboxylic acid cycle intermediates oxidized by NADP-linked enzymes support the greatest rate of steroid hydroxylations by adrenal mitochondria in vitro; e.g. NADP-isocitric dehydrogenase in rat adrenal[3] and malic enzyme in bovine adrenal[4] mitochondria. The adrenal gland of the gerbil, *Meriones unguiculatus*, secretes nearly equal amounts of 19-hydroxy-11-deoxycortisol and cortisol[5]. The 19-hydroxlating activity was shown to be localized, with the 11β-hydroxylase, in adrenal mitochondria where high rates of steroid hydroxylations in vitro occurred in the presence of isocitrate or malate[6]. In order to determine the source of intramitochondrial NADPH for steroid hydroxylation we studied the NADP vs NAD-linked dehydrogenation of isocitrate or malate and report gerbil adrenal mitochondria contain predominately NADP-isocitric dehydrogenase (NADP-IDH).

Materials and methods. Adrenal glands were removed from gerbils which had been allowed to expire in a carbon dioxide anesthesia chamber or from decapitated animals. Glands were homogenized at 4 °C in 0.25 M sucrose and 25 mM HEPES, pH 7.4 (buffered sucrose). Mitochondrial and microsomal fractions were obtained by differential centrifugation and washed by resuspension in buffered sucrose. Protein content was determined using the Bradford method[7]. Dehydrogenase activities were measured spectrophotometrically at 37 °C (340 nm, total volume 1.0 ml) in buffered sucrose with, as appropriate, 0.1–0.3 mM pyridine nucleotide, 7 mM $MgCl_2$, and 0.1–50 mM substrate. Steroid hydroxylation activities were determined using androstenedione (A) or testosterone (T) as precursor[6]. Steroids in the incubation media were extracted with dichloromethane and quantitated using High Performance Liquid Chromatography[8].

Table 1. Subcellular distribution of isocitric dehydrogenase and malate dehydrogenase in gerbil adrenal tissue

Fraction	Activity (nmoles/min/mg protein)*			
	Isocitric dehydrogenase NAD	Isocitric dehydrogenase NADP	Malate dehydrogenase NAD	Glucose-6 phosphate dehydrogenase NADP
Experiment 1				
Homogenate	–	480	2480	102
Nuclei/cell debris	–	118	934	–
Mitochondria	13	120	1220	–
+ ADP	20			
Microsomes	–	35	0	–
Cytosol	–	526	1220	160
Experiment 2				
Fresh mitochondria	19	43		
+ ADP	52	32		
Sonicate				
1 × 30 sec	20	154		
2 × 30 sec	64	206		
Freeze/thaw	–	247		

* The data presented for experiments 1 and 2 are results of replicate assays carried out on separate preparations of adrenal tissue. Enzyme activities in experiment 1 represent total activities determined after disruption of fractions by sequential sonication (30 s sonication on ice followed by 30 s cooling)